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STRATEGIES TO INCREASE HIGHLY-UNSATURATED OMEGA-3 FATTY ACIDS IN RAINBOW TROUT FED CANOLA, FLAX AND CAMELINA OILS

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Strategies to increase highly-unsaturated omega-3 fatty acids in rainbow trout fed canola, flax and camelina oils

Final Report

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Summary

The replacement of fish oil with vegetable oils in aquaculture diets reduces the levels of the desirable fatty acids eicosapentaenoic acid (20:5n-3; EPA) and docosahexaenoic acid (22:6n-3; DHA) and increases the level of the undesirable fatty acid arachidonic acid (C20:4n-6; AA). This project investigated methods to increase the levels of EPA and DHA and decrease the level of ARA in fish fed diets containing flax, canola or camelina oil replacing fish oil.

Milestone 1

Milestone 1 investigated the use of high levels of antioxidants added to flax, canola and camelina oils to improve the levels of EPA and DHA in rainbow trout. In this trial, rainbow trout were fed 4 oils (fish, canola, flax and camelina oils) with and without the addition of antioxidants (Vitamin E and butylated hydroxytoluene). The addition of antioxidants significantly increased the conversion of alpha-linolenic acid into EPA and DHA in fillets from rainbow trout. This indicates the importance of maintaining oil oxidative quality when producing oils for use in aquafeed production.

Milestone 2

The second Milestone investigated the addition of coriander oil to flax, canola and camelina oils to reduce the production of inflammatory fatty acids, such as AA and increase the production of the desirable fatty acids, EPA and DHA. Rainbow trout were fed diets containing the same 4 oils used in Milestone 1 (fish, canola, flax and camelina oils). The oils all contained antioxidants and therefore had high oxidative stability indices. The oils were formulated into an identical basal diet with and without the addition of 5 g/kg of coriander oil. The fish fed the diets containing coriander had

significantly higher levels of EPA and DHA than the fish fed the control diets. The levels of AA was decreased by over 20% but this difference was not statistically significant. These results indicate that coriander oil can be effectively used to enhance the conversion of alpha linolenic acid present in canola, camelina and flax oils to EPA and DHA. This increases the value of these oils in aquafeeds.

Abbreviations: ALA; alpha linolenic acid, EPA; eicosapentaenoic acid, DHA; docosahexaenoic acid, AA; arachidonic acid

Introduction

Aquaculture is currently growing at an average annual rate of 6.5% and this growth rate is expected to continue until 2025. This explosive growth in aquaculture has resulted in a concomitant growth of aquaculture feed production. Historically, aquaculture feeds have been based on two primary feed ingredients: fishmeal and fish oil, both of which have limited supply. The world's demand for fish oil already exceeds supplies and by 2020, the same will be true for fishmeal. The imminent scarcity of adequate supplies of fish oil amplifies the necessity of finding a replacement for fishmeal and oil. Until this dilemma is solved, the sustainability of aquaculture is in jeopardy.

Fish oil has a unique fatty acid composition. It has a high content of long chain polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (20:5n-3; EPA) and docosahexaenoic acid (22:6n-3; DHA). These fatty acids are important for the proper growth and intermediary metabolism of aquaculture species. It is difficult to simply

replace fish oil with another vegetable-based oil, as they lack EPA and DHA. Some fish have the capability to convert ALA to EPA and DHA, although in previous research, it has been shown that replacing fish oil with vegetable oil at high levels results in fish containing lower tissue EPA and DHA concentrations. Maintaining high levels of EPA and DHA in fish is important as health-conscious consumers desire high levels of these PUFAs in their diets for their health benefits. Thus, the replacement of fish oil with vegetable oil while maintaining the composition of aquaculture fish products is the central problem in aquaculture nutrition today.

Recent work in our laboratory has shown that the addition of high levels of antioxidants (Vitamin E and Butylated hydroxytoluene) to flax oil resulted in EPA and DHA levels in trout fillets that were similar to fish fed fish meal (Collins et al., 2007). Our hypothesis is that preventing the oxidation of flax oil in pelleted feeds results in more efficient conversion of ALA to EPA and DHA. We also noted increased levels of arachidonic acid in fish fed flax oil stabilized with antioxidants. This is undesirable since arachidonic acid is a precursor for inflammatory prostaglandins and leukotrienes.The production of arachidonic acid can be significantly reduced by feeding the omega-12 fatty acid petroselinic acid. Coriander oil is a rich source of petrosenlinic acid and is a crop grown in Saskatchewan.

This research proposal will answer two questions: 1) Can we increase the levels of EPA and DHA in salmonid fish fed vegetable oils high in ALA (linseed, canola and camelina oils) by using high levels of antioxidants and 2) Can we decrease the production of arachidonic acid by feeding coriander oil to with these three target oils.

Project Objectives

Overall Objective: To develop methods to improve the fatty acid composition of aquaculture fish fed vegetable oils instead of fish oils

Specific Objectives:

1) To investigate the use of high levels of antioxidants to increase the production of EPA and DHA in rainbow trout fed canola, flax and camelina oils.

2) To investigate the use of coriander oil to reduce the production of arachidonic acid and increase the levels of EPA and DHA in rainbow trout fed canola, flax and camelina oils.

Milestones

Milestone 1) Effect of antioxidant addition to canola, flax and camelina oils fed to rainbow trout:

Production of oils and diets

A Komet vegetable oil press was used to make the canola, flax and camelina oils. It involves selected seed being passed through a corkscrew shaped press and forced against a selected dye, which then extracts the oil and separates it from the press cake or meal. The oils were collected into brown bottles under nitrogen gas. After all the oils were produced they were divided into 2 aliquots and Vitamin E (5 g/kg) and butylated hydroxytoluene (BHT; 4 g/kg) were added to one aliquot of each vegetable oil and the fish oil. The oils were stored at room temperature until used.

The oils were incorporated into diets for the feeding experiment shown in Table 1. The diets differed only in the lipid source and contained 15.1 MJ/kg digestible energy and 378 g/kg of digestible crude protein. The diets met or exceeded the all other nutrient requirements of rainbow trout (NRC, 1993). The diets were cold extruded using a 3 mm die on a Hobart mixer. Following extrusion, the diets were dried in a forced air oven (55°C, 12 hours), chopped and screened to obtain a uniform pellet size.

Fish management

Triploid rainbow trout (*Oncorhynchus mykiss*) were purchased from Wild West Steelhead, Lucky Lake, SK, Canada and maintained in 360 L tanks that were part of a semi-closed recirculation system filtered biologically. The fish were fed to satiety twice daily and the amount of feed consumed by each experimental unit was recorded on a daily basis. Water temperature was maintained at 15 ± 1° C. Dissolved oxygen, pH and temperature were observed and recorded daily. Chlorine, nitrate, nitrite and ammonia were monitored on a weekly basis. Photoperiod was a 14 h light/10 h dark cycle. Animal protocols were approved by the University of Saskatchewan Committee on Animal Care and Supply, and followed principles established by the Canadian Council on Animal Care (1993). At the end of the 168 d feeding period, two fish per tank were filleted and the fillets stored at -20 °C.

Fatty acid analysis

Diets and fish carcass were analyzed for their fatty acid profile (O'Fallon et al., 2007). The fatty acids analyzed in this study were, linoleic acid (C18:2n-6), oleic acid (C18:1n-9), linolenic acid (C18:3n-3), arachidonic acid (C20:4n-6), eicosapentaenoic acid (C22:5n-3) and docosahexanoic acid (C22:6n-3). These fatty acids were selected for

analysis as they are the 6 major fatty acids found in plant and fish oils. Carcass and diet fatty acid composition was determined by gas chromatography using an Agilent 6890 system with Agilent.

ChemStation software (Agilent Technologies, Mississauga, ON). samples were skinned and de-boned prior to analysis. Samples were uniformly ground using a food processor (Moulinex DPA2, France) in the frozen state, and diets were ground through a 1mm mesh screen. Direct fatty acid methylation was then performed according to the procedure described by O'Fallon et al. (2007), with few minor differences. The samples were vortex-mixed using a single tube vortex instead of a multi-tube vortex, and samples were centrifuged (Beckman Coulter J6-MC Centrifuge, Mississauga, ON) for 5 minutes at 1500 rpm. Non-methylated C13:0 (Sigma-Aldrich, Inc., St. Louis, MO) was used as the internal standard, and all other chemicals used were of GC grade, and obtained from Sigma-Aldrich, Inc. (St. Louis, MO). Pressurized helium, air and hydrogen were purchased from Praxair Canada Inc. (Mississauga, ON). Fatty acid methyl ester samples were then compared to a Supelco 37 FAME mix (Cat# 47885) using the following GC program, slightly modified from O'Fallon et al. (2007): The machine was set for a 1.0 μl injection, split at a ratio of 20:1. The injector set points were a temperature of 260°C, pressure of 25 psi and a total flow of 23.4 ml/min. The initial oven temperature was set to 130°C and held for 1 minute. The temperature was ramped up at a rate of 20°C per minute to a maximum of 240°C and held for 25 minutes. The total run time for analysis was 54 minutes. The stationary phase was a Supelco fused silica capillary column SP 2560 (Sigma-Aldrich, St. Louis, MO).

Statistical Analysis

The experiment was analyzed as a 4 x 2 factorial design with 4 levels of oils (fish, flax, canola or camelina) and 2 levels of antioxidant (none or Vit E/BHT) using the general linear models procedure of SPSS

Table 1. Formulations and calculated analysis of diets used in the feeding experiment. The 8 diets were identical except for the inclusion of different oil sources (camelina, canola, fish or flax oil).

Ingredient	g/kg
Oil Product (fish, camelina, canola or flax oil)	127.66
Dicalcium Phosphate	8.55
DL-Methionine	0
Soybean meal	218.05
Soy protein concentrate	61.38
Corn Gluten Meal	120.36
Wheat	150
Canola Protein Concentrate	200
Vit/Min Premix	10
Choline Cl	4
Meat and bone meal	100

Digestible Nutrients	(%)
Phosphorus	1.10
DE Trout (kcal/kg)	4,200
Crude Protein	38.62
Methionine	0.74
Cysteine	0.68
Met+Cys	1.33
Lys	1.89
Thr	1.46
Arg	2.56
Ile	1.66
Val	1.96

Results

Oil oxidative stability

The addition of antioxidants to canola, flax and camelina oils more than doubled the oxidative stability indices of the oils after incorporation into diets (Table 2). This demonstrates that the antioxidants are still active in feed after processing.

EPA and DHA levels are decreased when fish oil is replaced by any of the 3 plant oils

The diets containing canola, flax or camelina oils were completely devoid of the long-chain unsaturated fatty acids including AA, EPA and DHA. The diets containing fish oil had significant amounts of EPA and DHA (Table 3). This indicates that the AA, EPA and DHA present in fillets from fish fed the vegetable oil diets are produced from conversion of LA and ALA present in canola, flax and camelina oils. The levels of EPA and DHA in fillets from fish fed the vegetable oil diets were significantly lower ($P < 0.05$) than in the fillets from fish fed the fish oil diet (Table 4).

Improved oil oxidative stability increases the levels of EPA and DHA in fish fed the 3 plant oils

The addition of antioxidants to the oils resulted in a significant increase in DHA in trout fillets. The level of EPA was also increased by the addition of antioxidants although the increase was not significant ($P > 0.05$; Table 4). These results confirm our previous work that the addition of the antioxidants Vitamin E and Butylated hydroxytoluene to flax increases the conversion of ALA to EPA and DHA. It also demonstrates that this approach works for canola oil and camelina oil.

Table 2. Oxidative stability indices of the feeds at the start of the feeding experiment. Values indicate the number of hours to completely oxidize a sample under controlled conditions.

Oil	No Antioxidant	Antioxidant
Fish	9.25	10.40
Canola	12.80	20.95
Flax	3.79	8.57
Camelina	4.48	12.35

Table 3. Fatty acid composition of diets (% of total fat).

Oil	Oleic Acid	Linoleic Acid	Linolenic Acid	Arachidonic Acid	EPA	DHA
Fish oil	16.65	6.35	2.75	0.90	4.93	9.90
Canola oil	58.10	18.30	8.50	0.00	0.00	0.00
Flax oil	20.90	16.10	48.10	0.00	0.00	0.00
Camelina oil	17.35	17.35	30.10	0.00	0.00	0.00
SEM	2.235	1.175	2.281	0.335	0.785	1.112
Antioxidant	28.65	14.83	22.28	0.30	1.61	2.55
No Antioxidant	27.85	14.23	22.45	0.34	1.85	2.40
SEM	0.532	0.461	0.249	0.111	0.291	0.230

Table 4. The effect of oil source and addition of antioxidants on the fatty acid composition of fish fillets from rainbow trout. Values are % of total fat.

Main Effects

Oil	**Oleic Acid**	**Linoleic Acid**	**Linolenic Acid**	**Arachidonic Acid**	**EPA**	**DHA**
Fish oil	28.0	9.2a	2.5a	0.6ab	4.0b	13.7c
Canola oil	32.3	12.8b	5.3ab	0.3a	2.7a	8.9a
Flax oil	24.5	12.1b	13.4c	0.8b	3.1a	10.3b
Camelina oil	23.5	11.2b	6.8b	0.6ab	3.1a	11.3b
Antioxidant						
No Antioxidant	25.9	10.5	8.2b	0.6	3.2	10.8a
Antioxidant	27.8	12	5.2a	0.6	3.6	12.1b
Pooled SEM	0.90	0.32	0.76	0.05	0.13	0.18
P-Values						
Oil	0.14	0.03	< 0.01	< 0.01	0.03	< 0.01
Antioxidant	0.21	0.17	0.01	0.45	0.11	0.03
Interaction	0.20	0.31	< 0.01	0.65	0.72	0.69

Milestone 2) Effect of coriander oil addition to canola, flax and camelina oils fed to rainbow trout on fillet fatty acid composition.

Production of oils and diets

A Komet vegetable oil press was used to make the canola, flax, camelina and coriander oils as described in Milestone 1. After all the oils were produced they were divided into 2 aliquots. One sample was used with the addition of Vitamin E (5 g/kg) and butylated hydroxytoluene (BHT; 4 g/kg) and to the second sample Vitamin E (5 g/kg), butylated hydroxytoluene (BHT; 4 g/kg) and 5 g/kg of coriander oil were added.

The oils were incorporated into diets for the feeding experiment shown in Table 1. The diets differed only in the lipid source and contained 18.1 MJ/kg digestible energy and 378 g/kg of digestible crude protein. The diets met or exceeded the all other nutrient requirements of rainbow trout (NRC, 1993). The diets were cold extruded using a 3 mm die on a Hobart mixer. Following extrusion, the diets were dried in a forced air oven (55°C, 12 hours), chopped and screened to obtain a uniform pellet size.

Fish management

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and Supply, and followed principles established by the Canadian Council on Animal Care (1993). At the end of the 168 d feeding period, two fish per tank were filleted and the fillets stored at -20 °C.

Fatty acid analysis

Diets and fish carcass were analyzed for fatty acids as described in Milestone 1.

Results

High Quality Oils

The vegetable oils were all produced at the University of Saskatchewan under ideal conditions; addition of antioxidant and the removal of oxygen immediately post crushing. This resulted in oxidative stability indexes (OSI) that were all higher than 1 hour (Table 6), which, is the rule of thumb cut off for acceptable oil quality. This meant that all the oils were of high quality and the conversion of ALA to EPA and DHA would be maximized.

No effect on fish growth

The substitution of fish oil with camelina, canola or flax oils had no effect on fish growth performance (Table 7). This is essential for the acceptance of these oils in the aquaculture industry, especially for camelina oil, which, has not yet been tested in rainbow trout diets.

Coriander oil improves the fatty acid composition of vegetable oil-fed fish

The levels of EPA and DHA in fish fillets were significantly increased in the fish fed coriander oil compared to controls (Table 8). Arachidonic acid was decreased by over 20% (Figure 1), however, this difference was not statistically different. In follow up work

in Norway, we found that the rate of production of arachidonic acid was significantly reduced in coriander oil-fed fish.

Table 5. Formulations and calculated analysis of diets used in the feeding experiment. The 8 diets were identical except for the inclusion of different oil sources.

Ingredient	Inclusion rate (g/kg)
Oil source[1]	120.0
Coriander oil	0.5
Blood meal	32.0
Poultry meal	100.0
Pea protein concentrate	243.1
Canola protein concentrate	250.0
Corn gluten meal	150.0
Celite	50.0
Dicalcium Phosphate	37.5
DL - Methionine	2.4
Choline Chloride	4.0
Vitamin/mineral premix	10.0
Vitamin C	0.5
Analysis (g/kg)	
Dry matter	97.2
Acid ether extract	18.8
Crude protein	46.3
Ash	13.8
DE(MJ/kg)	21.8

[1]The 4 oil sources were flax, camelina and fish oil.

Table 6. Fatty acid content and oxidative stability indices of the diets fed in Experiment 2.

Diet	Petroselinic Acid	Oleic Acid	Linoleic Acid	Linolenic Acid	Arachidonic Acid	EPA	DHA	Oxidative Stability Index (h)
Camelina	0.00	27.93	22.23	15.32	3.59	3.38	4.77	20.8
Camelina + Coriander	4.47	29.05	21.15	13.37	3.48	2.76	4.66	14.5
Canola	0.00	45.07	18.25	5.59	0.60	3.06	5.61	35.6
Canola + Coriander	1.83	42.70	19.29	5.73	0.17	2.25	3.90	23.9
Fish	0.00	25.03	12.95	3.00	1.55	12.27	12.82	8.1
Fish + Coriander	5.13	27.69	14.36	2.73	1.43	10.01	10.02	5.7
Flax	0.00	25.30	17.05	25.21	3.56	3.09	4.19	6.2
Flax + Coriander	3.69	19.76	17.70	25.27	2.83	2.47	2.96	2.7

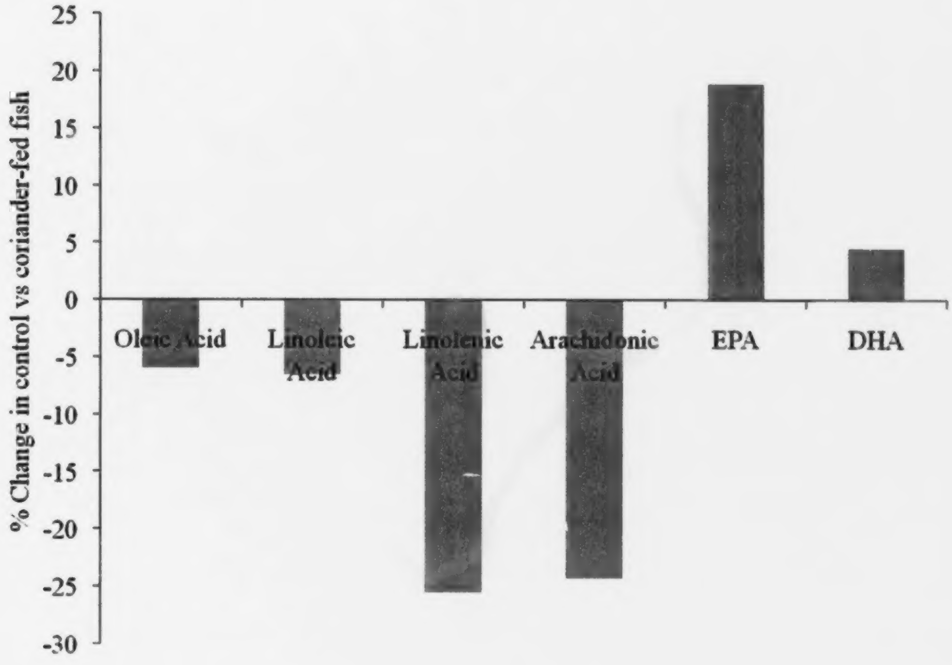
Table 7. Growth performance of fish fed diets containing camelina, canola, fish or flax oil with and without coriander oil

Oil Source	*SGR (%/d)*	*ADG (g/d)*	*Initial Weight (g)*	*Final Weight (g)*
Camelina oil	0.53	1.02	137.28	252.00
Canola oil	0.57	1.07	133.36	253.32
Fish oil	0.65	1.28	131.86	275.21
Flax oil	0.57	1.03	129.79	245.42
Pooled SEM	0.052	0.12	3.163	12.95
Coriander				
Control	0.57	1.13	137.93	264.62
Coriander	0.56	1.12	129.5	244.04
Pooled SEM	5.038	0.069	4.363	10.948
P-values				
Oil	0.269	0.337	0.309	0.41
Antioxidant	0.742	0.559	0.058	0.4
Interaction	0.875	0.854	0.454	0.782

Table 8. The effect of oil source and addition of antioxidants on the fatty acid composition of fish fillets from rainbow trout. Values are % of total fat.

Main Effects	Fatty Acid					
Oil	Oleic Acid	Linoleic Acid	Linolenic Acid	Arachidonic Acid	EPA	DHA
Camelina	28.3b	21.9c	14.7b	3.6b	3.2a	4.7a
Canola	44.3c	18.6b	5.7a	0.5a	2.8a	5.0a
Fish	26.7ab	13.9a	2.8a	1.5a	10.8b	11.0b
Flax	23.2a	17.3b	25.2c	3.3b	2.8a	3.7a
SEM	2.00	0.76	1.11	0.38	0.25	0.61
Coriander						
Control	29.21	18.24	14.49	2.73	4.95	6.28
Coriander	27.48	17.04	10.80	2.07	5.89	6.56
SEM	1.41	0.54	0.79	0.27	0.17	0.43
P-values						
Oil	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.069
Coriander	0.292	0.777	0.667	0.366	0.017	0.048
Interaction	0.322	0.370	0.724	0.810	0.969	0.195

Figure 1. Percent change of coriander-fed fish vs. controls in the levels of fatty acids in trout fillets.



Conclusions

The take home message from these studies is that we can improve the levels of EPA and DHA in trout fed vegetable oils. Oil quality is very important for maximizing these conversions, thus, feed grade canola, camelina and flax oils must still be handled to minimize oxidation. We can further improve the levels of EPA and DHA using coriander oil.

The major problem that must be addressed is the production of commercial quantities of coriander oil and an economical method to remove its scent. The fish fed coriander oil were strongly flavoured and this is unacceptable for commercial fish production.

We have established links with the Norwegian University of Life Sciences and Norwegian feed companies to test these approaches in salmon production. The graduate student who did the work described in this report spent 4 months in Norway performing trials to examine the mode of action of coriander oil in increasing EPA and DHA in rainbow trout. These links will hopefully result in markets for Saskatchewan grown oilseeds.

Other

Publications

K. M. Randall and M. D. Drew, 2010. Strategies to increase highly unsaturated omega 3 fatty acids in rainbow trout fed canola, flax and camelina oils. Proceedings of the International Symposium on Fish Nutrition and Feeding. June 1-4, 2010. Qingdao, China.

Awards

Kyla Randall, the graduate student on this project, won a Young Scientist award for her presentation at the International Symposium on Fish Nutrition and Feeding. June 1-4, 2010. Qingdao, China.

Extension activities

- Presentation of results of this trial to Biomar, a large aquafeed producer in Norway, the UK and Chile.
- Collaborative research on coriander oil with Dr. Margareth Overland at the Norwegian University of Life Sciences (Jan –Apr, 2011)
- Discussions with EWOS, largest aquafeed company in Norway on this technology.
- With ADF's permission, this report will be forwarded to the Saskatchewan Canola Development Commission, the Saskatchewan Flax Development Commission, Canpressco (farmer owned camelina processing facility located in southeast Saskatchewan, Canada), and the Saskatchewan Herb and Spice Association.

Expense Statement

A financial statement will be sent by research services.